इंटरनेट मानक

## Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

# IS 10232 (2003): General Rules for the Preparation of Initial Suspension and Decimal Dilutions for Microbiological Examination of Foods [FAD 15: Food Hygiene, Safety Management and Other Systems]

"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

Act Number 22 of 2005
The Indian Parliament
RIGHT TO INFORMATION

Made Available By
Public.Resource.Org

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

BLANK PAGE

BRUTUM FULMEN

IGNORANTIA JURIS NON EXCUSAT

PROTECTED BY COPYRIGHT

**IS 10232: 2003**
**ISO 6887-1:1999**
**(Reaffirmed 2005)**

*भारतीय मानक*

# खाद्य पदार्थों के सूक्ष्मजैविकीय परीक्षणों के लिए प्रारंभिक सस्पेंशन एवं डेसीमल डाइल्यूशन तैयार के सामान्य नियम

## *( पहला पुनरीक्षण )*

*Indian Standard*

# GENERAL RULES FOR THE PREPARATION OF INITIAL SUSPENSION AND DECIMAL DILUTIONS FOR MICROBIOLOGICAL EXAMINATION OF FOODS

## *( First Revision )*

ICS 07.100.30

© BIS 2003

**BUREAU OF INDIAN STANDARDS**
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

*February* 2003

**Price Group 3**

Food Microbiology Sectional Committee, FAD 46

## NATIONAL FOREWORD

This Indian Standard (First Revision) which is identical with ISO 6887-1:1999 ' Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Food Microbiology Sectional Committee and approval of the Food and Agriculture Division Council.

This standard was originally published in 1982 based on the earlier version of the International Standard, namely, ISO 6887: 1983. This is being revised to align with the revised version, namely, ISO 6887-1: 1999 under dual numbering.

In the adopted standard certain terminology and conventions are not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

The technical committee responsible for the preparation of this standard has reviewed the provisions of ISO 7218:1996 'Microbiology of food and animal feeding stuffs — General rules for microbiological examination', which is referred to in the adopted standard and has decided that it is acceptable for use in conjunction with this standard.

In this adopted standard, the following additional information shall be treated as added:

| | |
|---|---|
| Clause **5.2.1.2** and **5.2.2.1** | For sterilization of the diluents, a temperature of 121°C for 15 minutes must be maintained at a corresponding pressure of 103 kN/m$^2$ (15 psi). |

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2:1960 'Rules for rounding off numerical values (*revised*)'.

IS 10232 : 2003
ISO 6887-1 : 1999

*Indian Standard*

# GENERAL RULES FOR THE PREPARATION OF INITIAL SUSPENSION AND DECIMAL DILUTIONS FOR MICROBIOLOGICAL EXAMINATION OF FOODS

*( First Revision )*

## 1 Scope

This part of ISO 6887 defines general rules for the aerobic preparation of the initial suspension and of decimal dilutions for microbiological examinations of products intended for human or animal consumption.

This part of ISO 6887 is applicable to the general case, except for products mentioned in ISO 6887-2.

## 2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this part of ISO 6887. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreements based on this part of ISO 6887 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

## 3 Definitions

For the purposes of this part of ISO 6887, the following definitions apply.

### 3.1
### initial suspension (primary dilution)

suspension, solution or emulsion obtained after a weighed or measured quantity of the product under examination (or of a test sample prepared from the product) has been mixed with a nine-fold quantity of diluent, allowing large particles, if present, to settle

NOTE See clause 5 and notes 1 and 2 of 9.1.

### 3.2
### further decimal dilutions

suspensions or solutions obtained by mixing a measured volume of the initial suspension (3.1) with a ninefold volume of diluent and by repeating this operation with further dilutions until a decimal dilution series, suitable for the inoculation of culture media, is obtained

**3.3**
**specific standard**
an International Standard or guidance document describing the examination of a specific product (or group of products) for the detection or enumeration of a specific microorganism (or group of microorganisms)

## 4 Principle

Preparation of the initial suspension (3.1) in such a way as to obtain as uniform a distribution as possible of the microorganisms contained in the test portion.

Preparation, if necessary, of decimal dilutions (3.2) in order to reduce the number of microorganisms per unit volume to allow, after incubation, observation of their growth or not (in the case of tubes or bottles) or colony counting (in the case of plates), as stated in each specific standard.

NOTE In order to restrict the range of enumeration to a given interval, or if high numbers of microorganisms are foreseen, it is possible to inoculate only the necessary decimal dilutions (at least two successive dilutions) needed to achieve the enumeration according to the calculation described in ISO 7218.

## 5 Diluents

### 5.1 Basic materials

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the diluent, dehydrated basic components or a dehydrated complete preparation should be used. The manufacturer's instructions shall be rigorously followed.

Chemical products shall be of recognized analytical quality and suitable for microbiological analysis.

The water used shall be distilled water or of equivalent quality (see ISO 7218).

### 5.2 Diluents for general use

#### 5.2.1 Peptone salt solution

#### 5.2.1.1 Composition

| | |
|---|---|
| Enzymatic digest of casein | 1,0 g |
| Sodium chloride | 8,5 g |
| Water | 1 000 ml |

#### 5.2.1.2 Preparation

Dissolve the components in the water, by heating if necessary.

If necessary, adjust the pH so that, after sterilization, it is 7,0 ± 0,2 at 25 °C.

### 5.2.2 Buffered peptone water

#### 5.2.2.1 Composition

| | |
|---|---|
| Enzymatic digest of animal tissues | 10,0 g |
| Sodium chloride | 5,0 g |
| Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) | 9,0 g |
| Potassium dihydrogen phosphate ($KH_2PO_4$) | 1,5 g |
| Water | 1 000 ml |

#### 5.2.2.2 Preparation

Dissolve the components in the water, by heating if necessary.

If necessary, adjust the pH so that, after sterilization, it is 7,0 ± 0,2 at 25 °C.

## 5.3 Diluents for special purposes

See the specific standard appropriate to the product concerned.

NOTE ISO 6887-2 (under preparation) will give specific rules.

## 5.4 Distribution and sterilization of the diluent

Dispense the diluent (5.2 or 5.3) in volumes as necessary for the preparation of the initial suspensions into flasks (6.4) of appropriate capacity.

Dispense the diluent (5.2 or 5.3) in volumes as necessary for the preparation of the decimal dilutions into test tubes (6.5) or flasks (6.4) in quantities such that, after sterilization, each tube or flask contains 9,0 ml. The uncertainty of measurement of this final volume, after sterilization, shall not exceed ± 2 %.

NOTE If it is intended to count several groups of microorganisms using different culture media, it may be necessary to distribute all the diluents (or some of them) in quantities greater than 9,0 ml; the size of the flasks (6.4) and test tubes (6.5) being specified accordingly.

Stopper the tubes or flasks.

Sterilize in the autoclave at 121 °C for 15 min.

# 6 Apparatus and glassware

Usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following.

## 6.1 Apparatus for dry sterilization (oven) and wet sterilization (autoclave)

See ISO 7218.

## 6.2 Blending equipment

See ISO 7218.

## 6.3 Mechanical stirrer

See ISO 7218.

**6.4 Flasks or screw-cap bottles**, of appropriate capacities.

**6.5 Test tubes**, of appropriate capacities.

**6.6 Total-delivery graduated pipettes,** of nominal capacities 1 ml and 10 ml, graduated in 0,1 ml and 0,5 ml divisions respectively.

**6.7 pH-meter**, capable of being read to the nearest 0,01 pH unit at 25 °C, enabling measurements to be made which are accurate to ± 0,1 pH unit.

**6.8 Balance**, capable of weighing to the nearest 0,01 g.

## 7 Sampling

Carry out sampling in accordance with the specific standard appropriate to the product concerned. If such a specific standard is not available, it is recommended that agreement be reached on this subject by the parties concerned.

## 8 Preparation of test sample

See the specific standard appropriate to the product concerned. If such a specific standard is not available, it is recommended that agreement be reached on this subject by the parties concerned.

## 9 Procedure

### 9.1 Test portion and initial suspension (primary dilution)

Into a sterile bowl or sterile plastic bag, weigh, to a measurement uncertainty of ± 5 %, a mass $m$ g or measure, to a measurement uncertainty of ± 5 %, a volume of $V$ ml (minimum 10 g or 10 ml, unless otherwise stated) representative of the test sample (see clause 8).

Add a quantity of diluent equal to $9 \times m$ g or $9 \times V$ ml. This quantity can be measured preferably by mass with a measurement uncertainty of ± 5 % or by volume with a measurement uncertainty of ± 5 %.

NOTE 1 It may be necessary, in certain cases, particularly for products giving an initial 1 + 9 suspension which is too viscous or too thick, to add more diluent. This should be taken into account for subsequent operations and/or in the expression of results.

NOTE 2 This primary dilution partly conditions the value of the lower limit of enumeration, which also depends on the technique used (for example, pour-plate technique with a 1 ml inoculum of a 1/10 suspension, for which the limit is 10 microorganisms per gram). If it is necessary, for some enumerations in certain products, to fall below this limit, it is possible to use a smaller volume of diluent1). It should be noted that the inoculation of this initial suspension may result in difficulties due to inbalance in the inoculum/medium ratio (inhibition of the microbial growth by the increased concentration of the food components).

To avoid damage to the microorganisms by sudden changes in temperature, the temperature of the diluent during the operations given below shall be approximately the same as the ambient temperature, except for particular products (see specific standard).

Homogenize the mixture according to the recommendations of ISO 7218.

Allow large particles to settle, if necessary, for up to 15 min. Filtration systems giving equivalent results can be used.

---

1) In this case, the volume of diluent used should be reported in the test report.

In the case of enumeration of spores, a heat-treatment of the initial suspension, for example 10 min at 80 °C, shall be performed immediately after its preparation, followed by a quick cooling.

## 9.2 Further decimal dilutions

Transfer, by means of a pipette, 1 ml of the initial suspension with an uncertainty of measurement[2] of ± 5 %, into a tube containing 9 ml of sterile diluent at the appropriate temperature.

NOTE If a larger volume is necessary, it is possible to add a determined volume (more than 1 ml) of the initial suspension, with an uncertainty of measurement of ± 5 %, into a tube containing the nine-fold volume of sterile diluent.

For optimal precision, do not introduce the pipette more than 1 cm into the initial suspension.

Avoid any contact between the pipette containing the inoculum and the sterile diluent.

Mix thoroughly, preferably by using a mechanical stirrer (6.3) for 5 s to 10 s, to obtain a $10^{-2}$ dilution.

If necessary repeat these operations using the $10^{-2}$ and further dilutions by using at each dilution a new sterile pipette to obtain $10^{-3}$, $10^{-4}$, etc., dilutions, until the appropriate number of microorganisms has been obtained (see clause 4).

## 9.3 Duration of the procedure

The time lapse between the end of the preparation of the initial suspension and the instant when the inoculum comes into contact with the culture medium shall not exceed 45 min while limiting to 30 min the lapsed time between the preparation of the initial suspension (9.1) and the beginning of preparation of the following decimal dilutions, unless otherwise specified in the specific International Standard.

NOTE If the ambient temperature of the laboratory is too high, these two maximum durations should be reduced.

---

[2] The retained measuring uncertainty of ± 5 % takes into account the present limits of currently used pipettes.

**Bureau of Indian Standards**

BIS is a statutory institution established under the *Bureau of Indian Standards Act*, 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

**Copyright**

BIS has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in the course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Director (Publications), BIS.

**Review of Indian Standards**

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

This Indian Standard has been developed from Doc : No. FAD 46 (1220).

**Amendments Issued Since Publication**

| Amend No. | Date of Issue | Text Affected |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

BUREAU OF INDIAN STANDARDS

Headquarters :

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110 002 Telegrams : Manaksanstha
Telephones : 323 01 31, 323 33 75, 323 94 02 (Common to all offices)

| Regional Offices : | | Telephone |
|---|---|---|
| Central | : Manak Bhavan, 9 Bahadur Shah Zafar Marg<br>New Delhi 110 002 | 2323 76 17<br>2323 38 41 |
| Eastern | : 1/14, C.I.T. Scheme VII M, V.I.P. Road, Kankurgachi<br>KOLKATA 700 054 | 2337 84 99, 2337 85 61<br>2337 86 26, 2337 91 20 |
| Northern | : SCO 335-336, Sector 34-A, Chandigarh 160 022 | 260 38 43<br>260 20 25 |
| Southern | : C.I.T. Campus, IV Cross Road, Chennai 600 113 | 2254 12 16, 2254 14 42<br>2254 25 19, 2254 13 15 |
| Western | : Manakalaya, E9, MIDC, Marol, Andheri (East)<br>Mumbai 400 093 | 2832 92 95, 2832 78 58<br>2832 78 91, 2832 78 92 |
| Branch | : AHMEDABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KANPUR. LUCKNOW. NAGPUR. NALAGARH. PATNA. PUNE. RAJKOT. THIRUVANANTHAPURAM. VISAKHAPATNAM. | |

Printed by Printograph, New Delhi